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RESEARCH ARTICLE

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SRAP marker reveals genetic diversity in tartary buckwheat in China

Abstract Sequence-related amplified polymorphism (SRAP) marker was employed to analyze genetic diversity of 10 accessions of tartary buckwheat selected from a wide geographical area in China. Of the total 30 primer combinations investigated, 26 could amplify clearly and consistently. They produced a total of 285 fragments, of which 235 (82.5%) were polymorphic bands. Among the 26 primer combinations, five could discriminate all the genotypes used in this study. Based on the molecular data, the genetic similarity coefficients varied from 0.61 to 0.78 and calculated using the NTSYSpc published by Nei and Li (1979). The cluster analysis revealed that the 10 accessions were better to be grouped into two major clusters at a similarity level of 0.69. Moreover, the accessions collected from the same province turned out to be grouped in the same cluster, which indicated some geographical relationships. It also proved that the SRAP marker system was useful in identification and genetic diversity analysis of tartary buckwheat.

Keywords genetic diversity, NTSYS-pc, SRAP marker, tartary buckwheat

1 Introduction

Tartary buckwheat (*Fagopyrum tataricum*), an ancient dicotyledon belonging to Polygonaceae family, is a native species to the Himalayan regions such as Nepal, India, Bhutan, and China (Wang and Campbell, 2004). It is naturally growing and is being cultivated as a crop, which is used as a staple food and folk medicine in the regions (Jiang and Xing, 1992).

Tartary buckwheat is receiving more and more attention nowadays, owing to its diverse utilities. Besides a higher nutritional value, tartary buckwheat plays an important role in medical care as a pharmaceutical plant, especially with a high antioxidant activity due to its flavonoid content (Oomah and Mazza, 1996). For example, its seed rutin content reaches 0.8%–1.7%, while common buckwheat contains only 0.01% seed rutin content. Moreover, the data discrepancy reaches approximately 100 times (Fabjan et al., 2003). It has not only remarkable effects of antihyperglycemia (Wang et al., 1992), mitigating diabetes (Nagasawa et al., 2003), and mitigating cardiovascular diseases (He et al., 1995) but also of anti-inflammatory activities (Guardia et al., 2001) and anticancer activities (Park and Park, 2004).

The evaluation of genetic background of germplasm is essential to plant breeding. However, tartary buckwheat is predominantly a self-pollinated species, and it has difficulties in crossing the tiny flowers (2–3 mm) to obtain hybrids. Thus, the traditional measures are defeated by the molecular methods.

In recent years, several molecular techniques have been used for marker development, identification, and genetic analysis. Sequence-related amplified polymorphism (SRAP), reported by Li and Quiros (2001), possesses merits of simplicity, reliability, and moderate number of bands. Furthermore, their coding sequences can be targeted in the genome. SRAP has been successfully used for comparative genomics analysis (Li et al., 2003), evaluation of genetic diversity (Ferriol et al., 2003; Riaz et al., 2004), and map construction (Lin et al., 2003).

In this study, we applied SRAP to analyze genetic diversity of tartary buckwheat employing 30 primer combinations and 10 tartary buckwheat accessions. We want to provide evidence for tartary buckwheat hybrid breeding and genetic map by evaluating the primers (in the number of amplified fragments, the proportion of polymorphic bands, and the ability to discriminate accessions)

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and the genetic distance and diversity between tartary buckwheat strains (in the Nei genetic similarity coefficients and cluster analysis).

2 Materials and methods

2.1 Materials

We sampled 30 accessions of tartary buckwheat in a widely geographical area throughout China and grouped them into 10 major clusters based on 20 morphologic traits. Then, one representative accession from each cluster was selected. It turned out that the 10 accessions (Table 1) came from 6 provinces in China.

Seeds of each accession were sowed and grown in a growth chamber under a 16-hour photoperiod regime at room temperature. Two-week-old plants were harvested for DNA extraction.

Table 1 Accessions of tartary buckwheat used in the study

No.	accession	color of seed coat	shape of seeds	source
1	Heifeng-1	black	olivary	Shanxi
2	Fenghuang	brown	olivary	Hunan
3	Hunan 3-1	brown	triangular	
4	Hunan 7-2	black	cordiform	
5	Ding 98-1	motley	olivary	Gansu
6	Zhenba-II	brown	olivary	Shaanxi
7	YT-37	gray	olivary	Yunnan
8	Dianning-1	gray	triangular	
9	Kunminghui	gray	olivary	
10	Liuqiao-1	gray	triangular	Guizhou

2.2 DNA extraction

Total DNA was extracted by CTAB method (Li et al., 2007). The integrity and quality of the DNA were evaluated by electrophoresis on 0.8% agarose gel.

2.3 SRAP analysis

Primer pairs used in this study were synthesized by Shanghai Sangon Biological Engineering Technology and Service Co. Ltd. (Table 2). The PCR reaction mixtures (20 μL total volume) consisted of 0.25 mmol·L^{-1} dNTPs, 0.3 μmol·L^{-1} primers, 2.0 mmol·L^{-1} Mg^{2+}, 1×Taq buffer, 1 U Taq DNA polymerase (MBI), and 4 ng DNA.

Amplification were carried out in a PTC-0200 (BIO RAD) with the following PCR procedure: predenaturing at 94°C for 5 min, followed by five cycles of denaturing at 94°C for 1 min, annealing at 35°C for 1 min and elongation at 72°C for 1 min. In the following 35 cycles, the annealing temperature was increased to 50°C, with a final elongation at 72°C for 5 min. PCR products were resolved by electrophoresis on 6% denaturing acrylamide gels in 1×TBE buffer stained with silver photographed in SYNGENE Automated Gel Documentation System.

Table 2 The primer sequences of SRAP used in this experiment

primer	sequence (5′–3′)
Me1 (forward)	TGAGTCCAAA CCGG ATA
Me2 (forward)	TGAGTCCAAA CCGG AGC
Me3 (forward)	TGAGTCCAAA CCGG ACC
Me4 (forward)	TGAGTCCAAA CCGG TAG
Me5 (forward)	TGAGTCCAAA CCGG TGT
Em1 (reverse)	GACTGCGTACG AATT TGC
Em2 (reverse)	GACTGCGTACG AATT GCA
Em3 (reverse)	GACTGCGTACG AATT AGC
Em4 (reverse)	GACTGCGTACG AATT TAG
Em5 (reverse)	GACTGCGTACG AATT GGT
Em6 (reverse)	GACTGCGTACG AATT AAT

2.4 Data analysis

The presence or absence of each single fragment was coded by 1 or 0, respectively, and was scored for a binary data matrix, which was also used to calculate Jaccard's similarity coefficient. Cluster analysis (UPGMA) and dendrogram construction were performed with the SHAN program in NTSYS-pc software (Rohlf, 2000).

3 Results

The primers were selected based on previous reports of Li and Quiros (2001) and Ferriol et al. (2003). Thirty pairs of primers were used, which were combined with five forward primers by six reverse primers. Based on preliminary test, 26 sets of primers, which steadily produced clear amplification patterns, showed polymorphisms in all 10 genotypes.

Table 3 shows the data statistics of polymorphism obtained from 26 primer pairs. A total of 285 bands were observed. Among which, 235 were polymorphic (82.46%), ranging from 2 to 31 per primer combination, with an average of 9.04 bands per primer set. Twelve primer sets (Me2/Em6, Me3/Em1, Me3/Em5, Me3/Em6, Me4/Em2, Me4/Em4, Me4/Em5, Me4/Em6, Me5/Em3, Me5/Em4, Me5/Em5, and Me5/Em6) could reach a polymorphism rate of 100%.

The discrimination ability of the primers ranged from 20% to 100%, among which all the genotypes could be clearly distinguished by five primer sets (Me1/Em1, Me4/Em1, Me4/Em3, Me4/Em6, and Me5/Em6), accounting for 19.2% of the total primers.

Based on the calculational methods of Nei and Li

Table 3 The polymorphism from 26 primer combinations

No.	primer combination	allele point	polymorph-ism point	polymorph-ism rate/%	discrimina-tion ability/%
1	Me1/Em1	33	31	93.9	100
2	Me1/Em2	9	6	66.7	60
3	Me1/Em3	8	5	62.5	80
4	Me1/Em5	13	2	15.4	50
5	Me2/Em2	12	8	66.7	70
6	Me2/Em3	19	17	89.5	90
7	Me2/Em4	2	1	50	20
8	Me2/Em5	11	4	36.4	50
9	Me2/Em6	5	5	100	70
10	Me3/Em1	9	9	100	70
11	Me3/Em2	9	7	77.8	60
12	Me3/Em3	18	16	88.9	90
13	Me3/Em5	7	7	100	50
14	Me3/Em6	4	4	100	40
15	Me4/Em1	9	8	88.9	100
16	Me4/Em2	5	5	100	50
17	Me4/Em3	15	13	66.7	100
18	Me4/Em4	9	9	100	60
19	Me4/Em5	9	9	100	80
20	Me4/Em6	19	19	100	100
21	Me5/Em1	8	2	25.0	30
22	Me5/Em2	9	5	55.6	50
23	Me5/Em3	14	14	100	90
24	Me5/Em4	10	10	100	90
25	Me5/Em5	5	5	100	50
26	Me5/Em6	14	14	100	100

(1979), Jaccard's similarity coefficients (Table 4) gained from the SRAP data varied from 0.6105, which was between 'Ding 98-1' and 'Dianning-1', to 0.7825, which was among 'Kunminghui', 'Dianning-1', and 'Liuqiao-1'.

A dendrogram based on the similarity coefficients of the 10 genotypes was constructed (Fig. 1). The cluster analysis demonstrated that the 10 accessions were better to be grouped into two major clusters at a similarity level of 0.69. The accessions collected from the same province (Hunan and Yunnan) turned out to be grouped in the same cluster (I and II), which indicated some geographical relationships. The accessions from Shanxi and Gansu belonged to cluster I, while the accessions from Shaanxi and Guizhou belonged to cluster II.

4 Discussion

SRAP, a novel molecular marker, has been widely adapted for diversified purposes in different crops due to its simplicity, reasonable throughput rate, numerous codominant markers, and so on. Ferriol et al. (2003) researched into *Cucurbita pepo* with 11 SRAP primer combinations and identified a total of 88 reproducible fragments. Among them, 64 were polymorphic (72.70%). The number of polymorphic fragments for each primer combination varied from 1 to 14, with an average of 5.8. While in our study, we used 26 SRAP primer combinations to identify a total of 285 reproducible fragments. Among them, 235 were polymorphic (82.46%), and the number of polymorphic fragments for each primer combination varied from 2 to 31, with an average of 9.04, which showed that the polymorphism rate of this research was very high.

In the research by Guo and Luo (2006), 20 out of 72 primer combinations could be amplified clearly and consistently (27.8%) and just only one primer set, accounting for 5%, could reach 100% discrimination ability. However, in our study, the polymorphic primer set rate could reach 86.7% (26 out of 30 primer sets) and five primer combinations, accounting for 19.2%, could achieve 100% discrimination ability. Compared with other crops using SRAP, the results showed that SRAP was suitable to genomics analysis for tartary buckwheat in polymorphism rate and discrimination ability, etc.

With SRAP marker, Jaccard's similarity coefficients of 0.39–0.94 (Guo and Luo, 2006) and 0.63–0.88 (Qiao et al., 2007) were reported in *Diospyros kaki* Thunb and *Porphyra* lines, respectively. We obtained the coefficient

Table 4 The similarity coefficients of 10 accessions

No.	1	2	3	4	5	6	7	8	9	10
1	1.0000	–	–	–	–	–	–	–	–	–
2	0.7579	1.0000	–	–	–	–	–	–	–	–
3	0.7088	0.7263	1.0000	–	–	–	–	–	–	–
4	0.6982	0.7088	0.7509	1.0000	–	–	–	–	–	–
5	0.6807	0.6701	0.7333	0.7368	1.0000	–	–	–	–	–
6	0.6631	0.7579	0.7298	0.6982	0.7088	1.0000	–	–	–	–
7	0.6667	0.6772	0.6491	0.6526	0.6281	0.7158	1.0000	–	–	–
8	0.6842	0.6947	0.7018	0.6561	0.6105	0.6912	0.6737	1.0000	–	–
9	0.6912	0.7368	0.7158	0.7123	0.6807	0.7333	0.6596	0.7053	1.0000	–
10	0.6702	0.6947	0.6947	0.6561	0.6667	0.7193	0.6807	0.7825	0.7825	1.0000

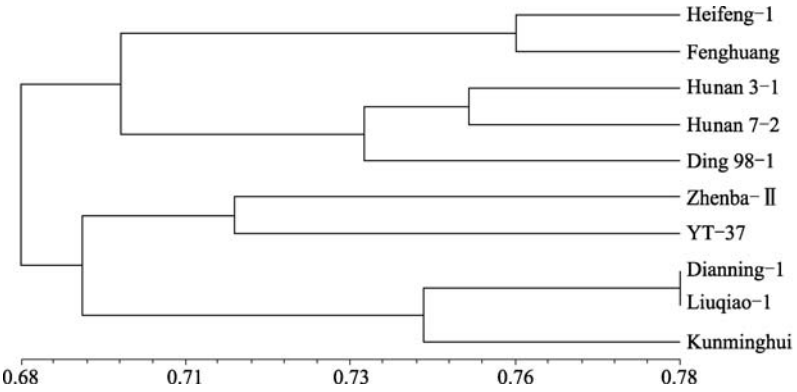

Fig. 1 An unweighted pair-group method with arithmetic averages (UPGMA) dendrogram

of 0.61–0.78 in tartary buckwheat. The relative moderate similarity of the buckwheat accessions in our study indicated the narrow genetic range of them. Moreover, extremely similar ones were eliminated in preliminary screening procedure based on their morphology.

In addition, SRAP is a new as well as a very useful molecular marker system. Its value of average discriminating power has been found to be the highest among the four systems (SRAP, SSR, ISSR, and RAPD) as reported by Budak et al. (2004). In our study, the SRAP amplification produced a lot of polymorphic bands, resulting in desirable discrimination ability. Our results first proved that the SRAP analytic system could be successfully used in the identification of the tartary buckwheat, providing evidence for tartary buckwheat hybrid breeding and genetic map, and making its contribution supplementary to the identification of tartary buckwheat using molecular methods.

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